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RESEARCH PAPER

Effect of Reproducibility of Nano-liquid Chromatography-Mass Spectrometry on Analysis of Urinary Peptidomics

WANG Yong[1,*], WU Li[2], XU Jin-Ling[2], LI Shui-Ming[1], LIU Ning[3], JIANG Liang[1]

[1] College of Life and Ocean Science, Shenzhen Key Laboratory of Marine Bioresources and Ecology, Shenzhen University, Shenzhen 518060, China
[2] College of Life and Ocean Science，Shenzhen Key Laboratory of Microbial Genetic Engineering, Shenzhen 518060, China
[3] Central Laboratory, The Second Hospital of Jilin University, Changchun 130041, China

Abstract: As an extension of proteomics, peptidomics has been widely used in medical and biological researches. However, the effect of reproducibility of identification method on peptidome is not clears. In this work, urine sample of a healthy people was analyzed for seven times in parallel by nano-liquid chromatography-high resolution tandem mass spectrometry (Nano-LC-MS/MS). To illustrate the variability and stability among these experiments, the number of spectra, the utilization of total spectra, the number of identified peptides, the number of proteins, and the ionic strength and retention time of peptides have been counted and compared. The average number of peptides was 208 and the standard deviation was 38.7. After all of data were combined, 426 peptides belonging to 114 proteins were obtained, while only 109 peptides coming from 35 proteins were identified in each experiment, indicating that there was both an randomness and a relative stability for LC-MS analysis. Increasing the number of parallel experiments would expand the data set of polypeptides, but the rate of increase would decrease after 3 or more measurements. In comparison with peptides, the results of peptidomics were more stable at protein level, indicating that proteins were more robust peptidomics biomarker than peptides.

Key Words: Peptidomics; Urine; Reproducibility; Random error

1 Introduction

The concept of peptidomics was proposed in the early twentieth century. Peptidome refers to all endogenous peptides in organisms such as body fluids, tissues and cells. The abnormal degradation products of proteins are related to various physiological and pathological processes. Peptidome is an important source for biomarkers[1–3], and exists in blood, urine, saliva, cerebrospinal fluid, sweat, tears and pleural effusion and other body fluids[4–12]. In addition, the studies on quantitative analysis of neuropically active peptides[13,14], cells[15,16], tissues[17,18], plant juices[19] and various labeled or unlabeled polypeptides have been reported[20–22].

Peptidomics is the extension and expansion of proteomics, in which peptides without specific enzyme digestion are separated and analyzed by mass spectrometric directly. Early study on the peptidome usually employed matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)[23] mass spectrometry which only gives the molecular weight information. Additionally, because of the ionization suppression effect, the detection of largest number of peptides is limited. Although TOF/TOF technology can be used to obtain more peptides and sequence information by the combination with LC-MALDI[24,25], this improvement is not very significant. In recent years, high-resolution mass spectrometry has been maturing, and its scanning speed has also been increased. And the combination of high-resolution mass spectrometry with liquid chromatography can greatly improve the ability of separation and analysis of peptides. Therefore nano-liquid chromatography-electrospray tandem

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*Corresponding author. Email: wyong@szu.edu.cn
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mass spectrometry has become an important analysis method for peptidome, which can not only be used to accurately identify the peptide sequence, but also be used to detect ladder sequence peptides as well as oxidized and phosphorylated peptides. In addition, quantitative labeling methods in proteomics have been applied in the analyses of peptides in recent years. It is worth noting that studies on reproducibility of mass spectrometry analysis in proteomics research have been reported recently. These studies report that the peptide level variability is higher than that of protein, and high resolution mass reproducibility is better than low resolution mass spectrometry analysis. In addition, the reproducibility of the results is related to the sample complexity, and the highest reproducibility of technical repetition is only 80%, while the lowest is only 35%[26]. However, to our knowledge, there has been no report on influence of reproducibility of analysis method on analysis results of proteomic yet. In this study, the same sample was repeatedly analyzed for 7 times in parallel to investigate the influence of reproducibility of the method on the experimental results under data dependent acquisition mode. The results showed that analysis results of this method were similar with that of proteomic analysis, and the reproducibility of LC-MS methods influenced identification of proteomic in some degree, especially for low abundance peptide and proteins.

2 Experimental

2.1 Instruments and reagents

Eksigentnano LC-Ultra™ 2D system (AB SCIEX), Triple TOF 5600 plus high resolution mass spectrometry (AB SCIEX), Protein Pilot 4.5 system (AB SCIEX), vacuum freeze-drying machine (Thermo savant) were used in this work.

Oxide graphene-lanthanum phosphate nanorods magnetic composite was synthesized according to literature method[1]. Mobile phase A of Nano liquid chromatography is 0.1% formic acid-2% acetonitrile, and mobile phase B is 0.1% formic acid-98% acetonitrile. C_{18} reversed-phase chromatography trap column and C_{18} reversed-phase chromatographic analytical column were used in liquid chromatographic analysis. All reagents of MS purity or guarantee reagent grade were purchased from Thermo Fisher Corporation (USA).

2.2 Reversed-phase chromatography-triple TOF mass spectrometry

Isolation and enrichment of polypeptides were performed according to the method as described in literature[1]. Seven aliquots of the above sample were simultaneously processed and the supernatant was collected and freeze-dried. The freeze-dried samples were then dissolved in buffer A of Nano-RPLC and rinsed on a C_{18} pre-column (100 μm × 3 cm, 3 μm, 15 nm) at a flow rate of 2 μL min^{-1}. In this work, the used nano-RPLC chromatograph was Eksigentnano LC-Ultra™ 2D system (AB SCIEX) and the analytical column was C_{18} reversed-phase column (75 μm × 15 cm, 3 μm 12 nm, ChromXPEksigent). Gradient elution program was as follows: 0–42 min, 5%–25% B; 42–56 min, 25%–40% B; 56–64 min, 80% B; 64–70 min, 5% B.

Mass spectrometric analysis of samples was performed on a TripleTOF 5600+ system (AB SCIEX) with nano-sprayed IIIion source (AB SCIEX, USA) under the optimized operation conditions, including air pressure of 2.4 kV, air curtain air pressure of 207 kpa, atomizing pressure of 34.5 kpa, and heating temperature of 150 ºC. Mass spectrum scanning was carried out in the collection mode of information dependence analysis (IDA). The mass spectrometry cycle was 3S. And for one full spectrum plus 30 tandem mass spectra, the precursor ions of the former top 20 with CPS > 300 were selected and analyzed by tandem mass spectrometry. The acquisition time of each MS/MS spectrum was 80 ms.

2.3 Data analysis

The original wiff files were submitted to the Protein Pilot Software v. 4.5 (AB SCIEX, USA) software for data processing and retrieval analysis. The database was a Homo sapiens race specific database in the uniprot library (containing 20210 protein sequences, download on January 2nd, 2015). The search parameters were set to non-digestion, phosphorylation and biological modification, and the false positive rate was controlled to be 1% FDR.

3 Results and discussion

3.1 Reproducibility analysis of identification results of peptidome

As shown in Fig.1, the total ion chromatograms of the seven analyses were similar, indicating that the results were reproducible. However, after searching the database, the total number of maps, the utilization rate of the map, the number of identified peptides, the number of proteins and the total ionic strength changed considerably (Table 1). Among them, the smallest amplitude of change in different measurements was the number of maps; the highest value was 1.29 times of the lowest value. The largest range of change was total ion flow strength, and the highest value was 3.96 times of the minimum value. The number of specific peptides varied by 1.72 folds, but the difference in protein detection was relatively small, which was 1.43 times. The relative standard deviation (RSD) of the number of peptides, proteins and total ion strength were 18%, 11% and 52%, respectively. The above results showed that the single analysis results of peptidome had certain